Title: **MICROTOX SOLID-PHASE TEST (SPT)**

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1.0 OBJECTIVE

This method measures the toxicity of materials that are tightly bound to particles in soil or sediment. The procedure allows Microtox® test organism , (*Vibrio fischeri*), to come in direct contact with toxicants in an aqueous suspension of the test sample, detecting both the soluble and insoluble organic and inorganic material.

2.0 HEALTH AND SAFETY

Personnel should wear lab coats, lab aprons, safety goggles, and chemical resistant gloves when preparing chemical stocks, and when dosing with test chemicals or effluents.

3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

This method should be restricted to use by or under the supervision of professionals experienced in toxicity testing.

4.0 REQUIRED AND RECOMMENDED MATERIALS

Microbics Model 500 Analyzer	Microtox® Test Reagent
Microtx® Reconstitution Solution	4-oz. Glass jar(s)
3.5% NaCl Diluent	White Tube Racks
Test Cuvettes	Lab coat
Gloves	Repeat Pipettor
0.6ml Syringe	Pipet Tips

7.5 ml SPT Tubes and Filters
500ml-Pipettor
50-ml Glass Beaker
1-L Volumetric Flask
Stir Bars
Aluminum weighing dishes
1000ml-Pipettor
250ml-Pipettor
De-ionized Water (DI H_2O)
1-5ml-Pipettor
Stainless steel spatulas

5.0 PROCEDURE

5.1 Preparation

5.1.1 Analyzer and Incubator Preparation

- Plug in the Microbics M500 Toxicity Analyzer.
- Place a cuvette in the REAGENT well.
- Plug in and turn on the incubator (water bath) and set it for 32.5°C on the dial (equivalent to 15°C for the test). Use a thermometer to verify the water temperature.

5.1.2 Making 3.5% NaCl Diluent

- Weigh out 35g of Microbiology Grade Sodium Chloride (located in Rm. 230) into a 50-ml glass beaker.
- Pour the NaCl into a 1-L volumetric flask.
- Add DI water to the mark on the neck of the flask.
- Cap with a stopper and invert to mix.
- Pour into a pre-clean 1000-ml Pyrex Culture Bottle.
- Date and label the bottle

5.2 Solid-Phase Test (SPT)

- If a Phenol test (SOP 00-???) is being conducted skip the next two steps.
- Turn water bath on and set for 32.5ºC on the dial (equivalent to 15ºC for test).
- Reconstitute reagent solution by adding 2-ml of Reconstitution Solution (water) to cuvette and placing in reagent well.
- Take sediment samples out of the walk-in freezer.
- Homogenize the sample. Place a 4-oz. Pre-cleaned glass jar (with a stir bar) on the scale and TARE the weight. Using a stainless steel spoon, weigh out 7.0 – 7.1g of sediment sample into the jar.
- Add 35ml of 3.5% NaCl diluent to the sediment and place the jar on a magnetic stir

plate. Stir the sediment so that it is well mixed.

- Place three sets of 10 SPT tubes into white racks in two rows of five.
- Add 1.5ml of SPT Diluent (3.5% NaCl) to each SPT tube, unless the initial concentration (IC) will be 19.737. If the IC=19.737%, then leave the last tube empty.
- If the IC=19.737%, then add 1.5ml of sediment directly into the last two tubes. If the IC=9.868% or lower, then add the 1.5ml of sediment to the last tube only.
- If the sediment is sandy then the IC will be 19.737%. The more clay and silt a sample has then the IC will be 9.868% or 4.934%. The finer the grain sizes of a sample, the lower the IC.
- With the sediment still stirring, use a 5ml-pipettor (set for 1.5 ml) and place the tip along the side of the jar. Making sure not to pipet up any debris, pipet 1.5ml into B5, D5, and F5.
- Make 1:2 dilutions by transferring 1.5ml from B5 to B4, Mix (3-4 times), B4 to B3, Mix (3-4 times), …A5 to A4. Mix. Discard 1.5ml from A4. The first three tubes are the controls.
- Repeat this for the other two replicates.
- Place tubes into 15ºC water bath on the hanging rack. Make sure the SPT tubes are still in the same order.
- Set timer for 10 minutes.
- During the 10 mins, reconstitute 2 vials of reagent. Try to get everything out of the vials.
- Mix reagent ~20 times with a 1-ml-pipettor.
- After 10 mins, mix reagent. Then transfer 20µl reagent to each SPT tube. Mix by hand. Set timer for 20 minutes.
- Place filters into SPT tubes. DO NOT push SPT filters down into the liquid.
- Set up computer for a **BASIC SOLID PHASE TEST:**

  3 CONTROL
  7 DILUTIONS
  3 SAMPLES
  (19.737, 9.868, 4.934, 2.467, or 1.234)= INITIAL CONC.
  2 = DILUTION FACTOR
  % = UNITS
  5 MINS: TEST TIME *Do not (Ö) the zero time reading box

- After 20 minutes, touch the space bar on the keyboard and begin sample transfers.
- For sample transfers, push the filter in the A1 SPT tube down into the liquid. DO NOT push filter down into the sediment. Transfer 500µl from SPT tube A1 to

cuvette A1, transfer 500µl from SPT tube A2 to cuvette A2 … transfer 500µl from SPT tube F6 to cuvette F6. Make sure to change pipette tips after transfer of B6 AND D6.

- Touch the space bar and wait for the remainder of test time to expire.
- At the end of the test time, place cuvette A1 in to the Read well and press the SET button. When the green light comes back on, READ cuvettes as prompted by the computer.
- Confidence Factor should be ≤ 2, R^2 should be ≥ 0.90, % Effect should bracket 50%.
- If the results deviate greatly from any of the above parameters, the sample(s) needs to be rerun.

5.3 End of the Test

Samples are considered to be environmental samples therefore they are can be disposed of in the sink and trash. The cuvette contents are disposed of in the sink and the cuvettes are thrown away in the BROKEN GLASS DISPOSAL by the door. The contents in the SPT tubes/filters are poured down the sink. The SPT tubes and filters are thrown away in the trash. The 4-oz jars are washed according to the Glassware SOP 00-???.

5.4 Correction of EC_{50} for Moisture

5.4.1 Determination of Sample Moisture Content

- It is recommended that the EC_{50} value be corrected to the weight of dry sample after the moisture content has been determined.

$$M = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} = \frac{\text{water weight}}{\text{wet weight}}$$

M = Moisture fraction

- In a fully ventilated area weigh out 20 g sample of soil in an aluminum weighing dish.
- Record wet weight.
- Place the dishes into a vented oven at 100°C for 16 hours.
- Weigh the dry sample and record the weight.
- Use the m value to correct the test results for water content. It is important to test the sample for toxicity ASAP after collection. DO NOT delay the Microtox® test while drying the sample, because the toxicity of the sample can change during the waiting time.

5.4.2 Correction for Moisture

There are two ways to correct the EC_{50} for moisture:

- Calculate the dry weight of the sample used, and calculate the actual concentration of the eluate on a dry sample basis. Re-enter the test data into the Microtox® software from the keyboard using the corrected concentration. This will calculate the corrected EC_{50} along with the other corrected statistical report information.

  Dry weight = wet weight – ((wet weight) (m))

- Correct the EC_{50} directly. This will correct the EC_{50} value, not he statistical report information.

  Corrected EC_{50} = EC_{50} – ((EC_{50}) (m))

6.0 QUALITY ASSURANCE/QUALITY CONTROL

Personnel should follow good laboratory practices during Microtox® testing.

7.0 REFERENCES

Microtox® Solid-Phase Protocols Manual. Microbics Corporation. 1992. Carlsbad, CA. 41 pp.